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Pharmacogenomics of Multigenic Diseases: Sex-Specific Differences in Disease and Treatment Outcome

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ABSTRACT

Numerous genetic variations have been shown to affect disease susceptibility and drug response. Pharmacogenomics aims at improving therapy on the basis of genetic information for each individual patient. Furthermore, sex chromosomes broadly determine biological differences between males and females. Consequently, substantial sex differences exist in phenotypic manifestation of disease and treatment response. This review discusses the role of sex in coronary artery disease, schizophrenia, and depression—complex multigenic disorders with considerable sex differences in frequency and presentation. Moreover, genetic factors underlying disease and drug response appear to differ between male and female patients. This appears to result at least in part from different physiological effects exerted by sex hormones such that polymorphisms in susceptibility genes may have physiological relevance only in males or females. However, few examples have been discovered to play a role in complex multigenic diseases, and the mechanistic basis of genetic variants as sex-dependent susceptibility factors has yet to be explored. Therefore, pharmacogenomic studies must consider sex differences in an effort to optimize individual drug therapy.

KEYWORDS: pharmacogenomics, sex differences, multigenic disease, candidate genes, coronary artery disease, depression, schizophrenia

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INTRODUCTION

Pharmacogenomics builds on the assumption that genetic variations between individuals contribute to disease susceptibility and drug efficacy or toxicity. Biological differences between males and females also contribute substantially to disease susceptibility and treatment outcome, although the responsible mechanisms are often overlooked and poorly understood. The term "sex" as used in this article refers to carriers of XY and XX chromosomes, representing males and females in a biological sense—notwithstanding commonly used physiological and anatomical criteria. These are indeed essential in cases of chromosomal abnormalities. "Gender," on the other hand, relates to cultural and acquired attributes of being male or female. In the literature these terms are often used interchangeably because regulatory agencies, elected officials, and others tend to avoid the use of the word "sex" in public writings.

THE BIOLOGY OF X AND Y CHROMOSOMES

The mammalian X and Y chromosomes are thought to have evolved from a common pair of autosomes.[1] Replacement of one X chromosome of 160 megabases (~3% of the complete human genome) with the distinctly different Y chromosome of only 60 megabases (~1%) results in pronounced genomic differences between males and females that overshadow most other genetic variations. Considering that genomic sequence of primates is said to be >98% identical to the human genome, the magnitude of male–female differences can be appreciated. The X chromosome has largely retained the original genes from its ancestor by evolutionary conservation including the process of crossovers (ie, exchange of chromosomal regions between 2 homologous chromosomes as compensation for deleterious mutations). This is possible in females (X-X) but not in males (X-Y). As a result, the Y chromosome has lost vast regions and holds only a limited set of genes (221 genes according to the National Center for Biotechnology Information http://www.ncbi.nlm.nih.gov/).

On the other hand, the Y chromosome, in the process of losing most of its original genes, has developed a highly conserved male specific region (MSY) comprising 95% of its length, and containing multiple copies of large palindromic sequences.[2] Many genes in the MSY are homologues to genes in autosomal regions, suggesting a mechanism of transposition of genes not present in X chromosomes. To compensate for lack of crossovers between homologous chromosomes, the Y chromosome appears to rely on gene conversion between repeat palindromic regions in the MSY as a means of conserving gene function in the absence of sexual recombination.[2] The MSY also harbors testes determining genes critical to male sexual development. Therefore, strong evolutionary forces preserve the current structure of the whittled-down Y chromosome. Limited regions of the X-chromosome are homologous to the remaining 5% of the Y chromosome, so that genes embedded in these areas behave as pseudo-autosomal genes allowing X–Y crossovers to occur.

Because the X chromosome exists in only one copy in males, X-linked mutations are fully expressed in males and are the cause of genetic diseases such as hemophilia or muscular dystrophy that mostly affect males. In females, the presence of 2 X chromosomes protects against the effects of recessive mutations but could potentially cause a harmful gene dosage effect if both copies of a gene were expressed at the same levels as in males. This effect is avoided by a mechanism of dosage compensation, a process of random silencing of a majority of either one of the 2 X chromosomes in every cell. As a result, gene expression may be mosaic at the cellular level: those heterozygous for mutations in X-linked genes express the defect in some cells and not in others. An additional genetic difference between males and females that becomes important during reproduction is the transfer of mitochondrial genes, which is restricted to mitochondria from the mother. A number of mitochondrial mutations lead to disease or are known to affect drug response or toxicity (for review see Chinnery and Turnbull).[3]

Overall, we need to consider the biology of X- or Y-linked genes, and their potential impact in males or females. Among the Y-linked genes, many are broadly expressed in multiple tissues and, therefore, are likely to define additional phenotypic differences between males and females. However, a large component of sex-based differences results from the secondary effect of sex hormones. Therefore, most known candidate genes conveying susceptibility to diseases with pronounced differences between males and females are autosomal. In the physiological context of male or female hormonal regulation, it appears plausible that polymorphisms in autosomal genes having no effect in one sex may contribute to disease susceptibility in the other sex. Absent a complete understanding of biological sex differences, we must design clinical studies such that sex does not represent a confounding issue, but rather the target for understanding biological differences. This review not only highlights the need to consider sex differences in pharmacogenomics but also reveals the dearth of specific genetic information that could differentially guide therapy in male and female patients.

SEX DIFFERENCES IN COMPLEX DISEASE AND DRUG RESPONSE

Considerable work has already focused on sex differences in the response to drug treatments. Prominent examples include kappa opioid receptor agonists, which are effective analgesics in females but not in males.[4,5] Also, drug metabolism–related differences have been reported, as a function of life cycle, particularly in females going through puberty or menopause.[6,7] Here we wish to address the question of how genetic factors contribute to disease susceptibility and drug response in complex disorders that are known to vary considerably in male and female patients. We have selected coronary artery disease, depression, and schizophrenia, because a rich literature already exists on the etiology of these diseases, and sex differences are prominent but still poorly understood. Moreover, drugs used in their treatment comprise nearly half of the 10 top-selling drugs, with considerable health implications and economic impact. Emerging from immense efforts in drug discovery, current treatments are highly effective; however, a portion of patients fails to respond or even suffers toxicity. Each of these diseases is thought to involve a genetic component, implicating numerous candidate genes as susceptibility factors. Most likely, combinations of genetic variations in multiple genes cooperate to increase disease susceptibility or affect drug response. While any of these relatively common genetic variations alone has little impact (penetrance), polymorphic gene combinations could lead to disease but may vary among individual patients, likely with somewhat different clinical outcomes for each combination. Moreover, epistasis—the interaction of multiple genetic variants either in *cis* or *trans*—further modulates phenotypic effects of quantitative trait loci. Numerous confounding factors make it exceedingly difficult to determine which genes, or combinations of genes, contribute to complex disorders. Lastly, population admixture—differences in frequency and linkage

disequilibrium between multiple single nucleotide polymorphisms (SNPs) (haplotypes)—accounts for differences in disease and treatment outcome among ethnic populations.

Since coronary artery disease (CAD), depression, and schizophrenia present with different clinical manifestations and at different ages in males and females, we ask whether genetic variations underlying the disease process differ among male and female patients. Similarly, females and males are likely to differ by genetic determinants of drug efficacy and toxicity. The latter is the subject of pharmacogenomics.

CORONARY ARTERY DISEASE

CAD ranks among the main causes of death. Factors contributing to CAD include genetic predisposition, lifestyle, and other risk factors such as hypertension and diabetes, inflammation, and aberrant hemostasis. Over 250 genes are suspected to play a role in susceptibility to CAD.[8,9] **Table 1** presents a selection of proposed CAD susceptibility genes; however, many of these require further validation. Moreover, their penetrance remains to be resolved on a case-by-case basis. Altered lipid metabolism has emerged as a main therapeutic target. Statins, 3-hydrox-3-methyl-glutaryl CoA (HMG-CoA) reductase inhibitors, are cholesterol-lowering medications that have reached "blockbuster" status in CAD therapy. Whereas statins are highly effective drugs in lowering cholesterol, disease progresses nevertheless in a significant portion of patients, extolling high mortality and staggering economic cost. Response to treatment appears to depend at least in part on genetic differences. Pharmacogenetics-genomics focuses on genetic variations that affect drug response—either directly via genes and their proteins in contact with statins (eg, drug metabolizing enzymes and transporters) or secondarily by affecting pathogenic processes underlying CAD. Thus, while statin treatment may significantly reduce cholesterol load, disease progresses, suggesting that other physiological factors could play a main role in such patients. Despite the overriding importance of statin therapy today, genetic risk factors for CAD and determinants of response to statin therapy remain uncertain, as does the cause for differences in male and female patients. Ability to predict risk and response to statin therapy would have profound effects on disease prevention and discovery of alternative therapy in statin-resistant patients.

Significant differences exist between males and females both in CAD susceptibility and treatment outcome. On the one hand, CAD risk in women is often underestimated. Moreover, women experience and communicate symptoms of disease differently than men. These pronounced *gender* differences contribute to differential treatment of male and female patients, as to when CAD is diagnosed and how aggressive the treatment is (based on perceived risk).[19] There is a 2:1 prevalence of male over female patients in younger age groups, but this difference dissipates with increasing age. Moreover, sex differences exist in the clinical manifestations of CAD. Corollary risk factors, such as diabetes and blood pressure, differ dramatically in their impact on CAD between males and females.[20,21] There is evidence that genetic variations of some susceptibility genes have sex specific effects. In a recent large study of 112 polymorphisms in 71 candidate genes, involving unrelated Japanese CAD patients and controls, candidate genes identified as risk factors differed between male and female patients. Risk of myocardial infarction was significantly associated with SNPs in the connexin 37 gene in men and in the plasminogen-activator inhibitor type 1 and stromelysin-1 genes in women.[14] These genetic variants may prove to be useful predictors of genetic risk, but they also highlight biological differences in CAD between males and females. Whether the results of this study extend beyond the ethnic population studied remains to be determined.

Polymorphisms in candidate genes such as cholesteryl ester transferase (*CETP*) have differential effects on CAD in males and females.[22,23] For example, the TaqI B2 allele of *CETP* appears to be correlated with high-density lipoprotein (HDL) levels in males but not females and correlates with poor response to pravastatin; however, this was tested only in males.[24] It is possible that TaqI B does not play a significant role in treatment outcome for female CAD patients. As estrogens are associated with higher HDL-cholesterol (HDL-C) levels, it is likely that optimal *CETP* alleles with different effects on cholesterol-transfer ability differ between males and females. However, because of a perceived CAD prevalence in male over female patients, such studies have often excluded females. This is not to say that CAD is of lesser concern for women. Age of onset is higher in women than men, but mortality is nevertheless high. Estrogens may have protective effects (eg, by elevating HDL) but this may also depend upon genotype with respect to estrogen receptors.[25] The deleterious effects of estrogen need to be taken into account in hormone replacement therapy after menopause. Pharmacogenomic studies must account for clear differences between male and female CAD patients, because genetic risk factors and determinants of statin response are likely to differ substantially. Moreover, risk factors may differ by ethnicity, and this confounds the predic-

Table 1. Candidate Genes Involved in the Susceptibility to or Pharmacotherapy of Coronary Artery Disease*

Gene Name	Symbol	Chromosomal Location
Adenosine monophosphate deaminase 1	AMPD1	1p13.2
Advanced glycosylation end product-specific receptor	AGER	6p21.33
Aldosterone synthase	CYP11B2	8q24.3
Alpha adducin	ADD1	4p16.3
Alpha-1-antichymotrypsin	AACT†	6q16.1
Alpha-2A-adrenergic receptor	ADRA2A	10q25.2
Alpha-2B-adrenergic receptor	ADRA2B	2q11.2
Alpha-2-macroglobulin	A2M	12p13.31
Angiotensin converting enzyme	ACE	17q23.3
Angiotensin II receptor AT1	AGTR1	3q24
Angiotensin II receptor AT2	AGTR2	Xq23
Angiotensinogen	AGT	1q42.2
Apolipoprotein A	LPA	6q26-q27
Apolipoprotein A4	APOA4	11q23.3
Apolipoprotein B	APOB	2p24.1
Apolipoprotein E	APOE	19q13.32
Adenosine triphosphate binding cassette A1	ABCA1	9q31.1
Beta-1-adrenergic receptor	ADRB1	10q25.3
Beta-2-adrenergic receptor	ADRB2	5q32
Beta-3-adrenergic receptor	ADRB3	8p12
Beta-adrenergic receptor kinase 1	ADRBK1	11q13.3
Bradykinin receptor B2	BDKRB2	14q32.2
Brain natriuretic peptide precurser	NPPB	1p36.22
Cadherin 5	CDH5	16q22.1
Cathepsin G	CTSG	14q11.2
Caveolin 1	CAV1	7q31.2
Caveolin 2	CAV2	7q31.2
Caveolin 3	CAV3	3p25.3
CD24 antigen	CD24	6q21
CD36 antigen	CD36	7q21.11
Chemokine (CX3C) receptor 1	CX3CR1	3p22.2
Cholesterol 7 alpha-hydroxylase	CYP7A1	8q12.1
Cholesterol ester transfer protein	CETP	16q13
Collagen type III A1	COL3A1	2q32.2
Colony stimulating factor 1	CSF1	1p21-p13
Connexin 37 (gap junction protein alpha 4)	GJA4	1p34.3
C-reactive protein	CRP	1q23.2
Cystathionine beta-synthase	CBS	21q22.3
Dolichyl-diphosphooligosaccharide-protein glycosyltransferase	DDOST	1p36.12
Early growth response protein-1	EGR1	5q31.2
Endothelial nitric oxide synthase	NOS3	7q36.1
Endothelin 1	EDN1	6p24.1
Endothelin converting enzyme	ECE1	1p36.12
Endothelin receptor A	EDNRA	4q31.22
Endothelin receptor B	EDNRB	13q22.3
Estrogen receptor alpha	ESR1	6q25.1
Factor II (prothrombin)	F2	11p11.2
Factor V	F5	1q24.2
Factor XIII A1-subunit	F13A1	6p25.1
Fatty acid-binding protein 2	FABP2	4q26
Fibrinogen alpha	FGA	4q31.3
Fibrinogen beta	FGB	4q31.3
Fibrinogen gamma	FGG	4q32.1

Table 1. Candidate Genes Involved in the Susceptibility to or Pharmacotherapy of Coronary Artery Disease* (Continued)

Gene Name	Symbol	Chromosomal Location
GATA-binding protein 4	GATA4	8p23.1
Gelatinase B, matrix metalloproteinase 9	MMP9	20q13.12
Glucagon receptor	GCGR	17q25
Glutathione S-transferase mu	GSTM1	1p13.3
Glutathione S-transferase theta	GSTT1	12p12.3
Glycogen synthase	GYS1	19q13.33
G-protein subunit b3	GNB3	12p13.31
Granulocyte macrophage colony stimulating factor	CSF2	5q23.3
Haptoglobin	HP	16q22.2
Heparin sulfate proteoglycan	HSPG2	1p36.12
Hepatic lipase	LIPC	15q21.3
Hepatocyte growth factor	HGF	7q21.11
Histidine-rich glycoprotein	HRG	3q27.3
Hormone-sensitive lipase	LIPE	19q13.2
Inducible nitric oxide synthase	NOS2A	17q11.2
Insulin	INS	11p15.5
Insulin receptor substrate-1	IRS1	2q36.3
Insulin receptor substrate-2	IRS2	13q34
Insulin-like growth factor 1	IGF1	12q23.2
Integrin alpha 4	ITGA4	2q31.3
Integrin alpha L	ITGAL	16p11.2
Integrin alpha X	ITGAX	16p11.2
Integrin beta 1	ITGB1	10p11.22
Integrin beta 2	ITGB2	21q22.3
Inter cellular adhesion molecule 1	ICAM1	19p13.2
Interleukin 1 alpha	IL1A	2q13
Interleukin 1 receptor antagonist	IL1RN	2q13
Interleukin 10	IL10	1q32.1
Interleukin 2	IL2	4q27
Interleukin 6	IL6	7p15.3
Lectin galactose binding soluble 3	LGALS3	14q22.3
Leucocyte neutrophil elastase gene	ELA2	19p13.3
Lipoprotein lipase	LPL	8p21.3
Low-density lipoprotein receptor	LDLR	19p13.2
Low-density lipoprotein receptor-related protein	LRP1	12q13.3
Macrophage scavenger receptor	MSR1	8p22
Major histocompatibility complex: region HLA-DRB1*03	HLA-DRB1	6p21.3
Matrix Gla protein	MGP	12p12.3
Methionine synthase	MTR	1q43
Methionine synthase reductase	MTRR	5p15.31
Methylenetetrahydrofolate reductase	MTHFR	1p36.22
Microsomal triglyceride transfer protein	MTP	4q23
Monocyte chemoattractant protein receptor	CCR2	3p21.32
Monocyte differentiation antigen CD14	CD14	5q31.3
Myeloperoxidase	MPO	17q23.2
Natriuretic peptide precursor A	NPPA	1p36.22
Neuropeptide Y	NPY	7p15.3
Nuclear factor of activated T cell, cytoplasmic, 4	NFATC4	14q11.2
Osteocalcin	BGLAP	1q22
Osteopontin	SPP1	4q22.1

Table 1. Candidate Genes Involved in the Susceptibility to or Pharmacotherapy of Coronary Artery Disease* (Continued)

Gene Name	Symbol	Chromosomal Location
Oxidized low density lipoprotein receptor 1	OLR1	12p13.2
Paraoxonase 1	PON1	7q21.3
Paraoxonase 2	PON2	7q21.3
Platelet-derived growth factor receptor A	PDGFRA	4q12
Platelet-derived growth factor receptor B	PDGFRB	5q32
Peroxisome proliferator-activated receptor alpha	PPARA	22q13.31
Peroxisome proliferator-activated receptor gamma	PPARG	3p25.2
Plasma cell differentiation membrane glycoprotein	ENPP1	6q23.2
Plasminogen activator inhibitor 1	SERPINE1	7q22.1
Platelet-derived growth factor A	PDGFA	7p22
Platelet-derived growth factor B	PDGFB	22q13.1
Platelet endothelial cell adhesion molecule	PECAM1	17q23.3
Platelet-activating factor receptor	PTAFR	1p35.3
Platelet glycoprotein IIIa (PLA1)	ITGB3	17q21.31
Protein kinase C substrate, 80KD, heavy chain	PRKCSH	19p13.2
Protein phosphatase 1, regulatory inhibitor subunit 2	PPP1R2	3q29
Protein phosphatase 1, regulatory inhibitor subunit 3	PPP1R3A	7q31.1
Protein phosphatase 3, catalytic subunit, alpha isoform	PPP3CA	4q24
Protein phosphatase 3, catalytic subunit, beta isoform	PPP3CB	10q22.2
Purinergic receptor P2Y, G protein-coupled, 1	P2RY1	3q25.2
Selectin E	SELE	1q24.2
Selectin L	SELL	1q24.2
Selectin P	SELP	1q24.2
Selectin P ligand	SELPLG	12q23.3
Serotonin transporter	SLC6A4	17q11.2
Small inducible cytokine A2	CCL2	17q11.2
Sodium calcium exchanger	SLC8A1	2p23-p22
Stromelysin 1	MMP3	11q22.2
Thrombin receptor	F2R	5q13.3
Tissue factor	F3	1p21.3
Tissue factor pathway inhibitor	TFPI	2q32.1
Transforming growth factor beta 1	TGFB1	19q13.2
Tumor necrosis factor alpha	TNF	6p21.33
Tumor necrosis factor receptor superfamily, member 1A	TNFRSF1A	12p13.31
Tumor necrosis factor receptor superfamily, member 1B	TNFRSF1B	1p36.22
Tumor necrosis factor-alpha-induced protein 6	TNFAIP6	2q23.3
Vascular cell adhesion molecule 1	VCAM1	1p21.2
Vascular endothelial growth factor	VEGF	6p21.1
Very low density lipoprotein receptor	VLDLR	9p24.2
Vitamin D receptor	VDR	12q13.11

*Several publications were considered in compiling this list.[10-16] Chromosomal position was obtained from the GeneCards Web site.[17] The column labeled "Chromosomal Location" uses the following convention: the numbers 1 through 22 (or X) on the left refer to chromosome identity; "p" or "q" refers to the arm of the chromosome on which the gene is located; and the number on the right is the cytogenetic band.

†Abbreviation not yet approved by HUGO/GDB nomenclature committee.[18]

tive value of susceptibility genes among patient populations. Clearly, more work needs to be done to bring these differences into sharper focus, and thereby, understand the disease better, improve our ability to treat CAD early, or even prevent it altogether.

SEX DIFFERENCES IN MENTAL DISORDERS: DEPRESSION AND SCHIZOPHRENIA

Many disorders of the central nervous system have distinct sex differences in their manifestation. Anxiety, depression, eating disorders, and Alzheimer's disease are more common in women. Men, on the other hand, are more likely to be afflicted with alcohol and drug abuse problems, antisocial personality, attention deficit disorders, and Tourette's syndrome. Here we focus on 2 distinct disorders, depression and schizophrenia. Schizophrenia shows gender differences in the development of the disorder and affects men earlier in life and more severely, even though men and women both get the disorder at an equal rate. Depression, by contrast affects women more than men. These 2 diseases share putative susceptibility genes and metabolic pathways (**Table 2**). However, the pathological significance of most if not all polymorphisms in candidate genes remains to be confirmed. Only recently several genes have been shown to play a significant role in schizophrenia[31,39,49] and depression.[27,32,47,48,50] These genes include, but are not limited to, *COMT*,[49,51] *SERT*,[44] *neuregulin*,[52] and *DISC1*[39] for schizophrenia and *nNOS*,[47] *β-adrenergic receptor-1*,[48] *MTHFR*,[27] and *SERT*[27,32,47,48,50] for depression. For the most part, the differential impact of gene variants in male and female patients has yet to be studied. Many of these genes are listed as candidates for both disorders. The overlap between putative susceptibility genes for schizophrenia and depression must be interpreted with caution, as selection of candidate genes may be biased because investigators tend to focus on well-studied genes. Even though these diseases have opposite preference between women and men, the underlying genetic mechanisms that cause these disorders to be sex specific may be related.

DEPRESSION AND TREATMENT

Depression is a common condition that affects approximately 19 million Americans in any year. It can strike individuals equally across educational, economic, and ethnic boundaries. There are 3 frequent types of depression that vary in severity of symptoms and persistence: (1) major depression (also called unipolar depression) in which symptoms interfere with the ability to eat, sleep, work, and enjoy life; (2) dysthymia, which is long term or chronic but nondisabling; and (3) bipolar disorder, characterized by wide mood swings ranging from deep lows to manic highs. Epidemiological and clinical studies have consistently observed significant sex-specific differences among patients with depression, with females outnumbering males at a rate of 2:1. There is not a significant sex difference between the rates of depression in children, but differences become evident after onset of puberty.[53] In fact, the increase in the rate of depression of adolescent girls is correlated not to age, but to the physical changes that occur during puberty.[53] Premenstrual syndrome (PMS) and postpartum depression (PPD) are additional conditions involving depression that specifically affect women and are suggestive of hormonal involvement in the pathogenesis of mood disorders.

Depression is often treated with a combination of psychotherapy and medication. The most common antidepressants used in the treatment of depression are selective serotonin reuptake inhibitors (SSRIs), tricyclics, and monoamine oxidase inhibitors (MAOIs). Tricyclics work by inhibiting norepinephrine and serotonin reuptake. They also antagonize many neurotransmitter receptors, which may be the cause of their many side effects. SSRIs were developed to specifically target the serotonin transporter SERT, and MAOIs function by selectively inhibiting MAO enzymes. Most antidepressants are also effective in treating some anxiety disorders. There is variation in the response of an individual to any particular drug. Drug response rates vary from 85% for MAOIs to as low as 55% for SSRIs.[54] Candidate genes thought to play a role in susceptibility or drug treatment of depression and/or schizophrenia are listed in **Table 2**. Various candidate genes have been studied for variations that are correlated with antidepressant response.[55] The promoter region of serotonin transporter gene, *SERT*, which exists in 2 variants, long and short, has been examined by a number of groups. For example, patients who are homozygous for the short variant were found to respond better to fluoxetine (Prozac) than patients with other genotypes.[56] By contrast, patients homozygous for the short variant responded worse to fluvoxamine (another SSRI) than patients with other genotypes.[57] Any association study must further reflect environmental factors. For example, the *SERT* promoter polymorphism was significantly associated with depression only in individuals with stressful experiences in early life.[27,32,47,48,50] In a study to determine if men and women respond differently to antidepressants, women were found to have a

Table 2. Candidate Genes Involved in the Susceptibility to or Pharmacotherapy of Schizophrenia and/or Depression*

Gene Name	Symbol	Chromosomal Location	Schizophrenia	Depression
153 amino acid long predicted protein	G72†	13q33.2	+	-
Beta-1-adrenergic receptor	ADRB1	10q25.3	-	+
Brain-derived neurotrophic factor	BDNF	11p14.1	+	+
cAMP response element binding protein	CREB1	2q33.3	-	+
Catechol-O-methyltransferase	COMT	22q11.21	+	+
Ciliary neurotrophic factor	CNTF	11q12.2	+	-
D-amino acid oxidase	DAO	12q24.11	+	-
Disrupted in schizophrenia	DISC1	1q42.2	+	-
Dopamine receptor D2	DRD2	11q23.2	+	-
Dopamine receptor D3	DRD3	3q13.31	+	-
Dopamine receptor D4	DRD4	11p15.5	+	-
Dysbindin	DTNBP1	6p22.3	+	-
Estrogen receptor alpha	ESR1	6q25.1	+	+
Methylenetetrahydrofolate reductase	MTHFR	1p36.22	-	+
Monoamine oxidase A	MAOA	Xp11.3	-	+
Neuregulin 1	NRG1	8p12	+	-
Neuronal nitric oxide synthase	NOS1	12q24.22	-	+
Neuropeptide Y	NPY	7p15.3	+	-
Neurotrophin-3	NTF3	12p13.31	+	-
Norepinephrine transporter	SLC6A2	16q12.2	+	+
Notch 4	NOTCH4	6p21.33	+	-
Phospholipase A2	PLA2G1B	12q24.23	+	-
Proline dehydrogenase 1	PRODH	22q11.21	+	-
Reelin	RELN	7q22.1	+	
Regulator of G-protein signaling 4	RGS4	1q23.3	+	-
Retinoic acid inducible-1	RAI1	17p11.2	+	-
Serotonin receptor 1A	HTR1A	5q12.3	-	+
Serotonin receptor 1B	HTR1B	6q14.1	-	+
Serotonin receptor 2A	HTR2A	13q14.2	+	+
Serotonin receptor 2B	HTR2B	2q37.1	-	+
Serotonin receptor 2C	HTR2C	Xq23	+	+
Serotonin receptor 5A	HTR5A	7q36.2	+	-
Serotonin transporter	SLC6A4	17q11.2	+	+
Tryptophan hydroxylase	TPH1	11p15.1	+	+
Vesicular monoamine transporter type-2	SLC18A2	10q26.11	+	-

*Several publications were considered in compiling this list.[26-48] The + symbol indicates that a gene has been implicated; however, a causative link has yet to be established for most if not all genes. Chromosomal position was obtained from the GeneCards Web site.[17] The conventions used in the chromosomal location column are as described in the legend for Table 1.

†Abbreviation not yet approved by HUGO/GDB nomenclature committee.[18]

superior response to MAOIs compared with men, but no other differences were noted.[54] Again, our understanding of the genetic factors contributing to depression and drug response in male and female patients requires more study.

Estrogen has been shown to have antidepressant effects. Women with severe PPD or postpartum psychosis were reported to respond rapidly to oral estrogen treatment in some studies.[58-60] The speed by which therapy becomes effective is important because it may take several weeks for antidepressants to take full effect. Therefore, estrogen-induced accelerated responsiveness is a promising strategy in female patients under special conditions. High-dose oral estrogen has been administered as a prophylactic in women with history of severe postpartum affective disorder.[61] There

is some evidence that combining estrogen with traditional antidepressants to treat depression is effective. The effects of estrogen replacement therapy were examined in a randomized, double-blind trial of fluoxetine versus placebo in elderly depressed women. Women taking estrogen who were treated with fluoxetine improved significantly more than estrogen-treated patients who received a placebo.[62] Patients who did not receive estrogen showed no difference in response to fluoxetine versus placebo, suggesting that estrogen may boost the effects of SSRIs, perhaps by interaction with the serotonin system.[62] In such studies, polymorphisms in any of the genes contributing to disease or drug response have yet to be considered systematically. For example, polymorphisms in the estrogen receptor[63] may affect treatment outcome, as could polymorphisms in the serotonin transporter and further genes involved in neurotransmission. However, risk associated with estrogen use must be considered as well.

SCHIZOPHRENIA AND TREATMENT

Schizophrenia is a debilitating form of psychosis that affects approximately 1% of the population. Although the exact mechanism of pathogenesis is unknown, excessive activity at dopaminergic synapses in the brain is thought to play an important role.[64] The onset of schizophrenia coincides with reproductive years and hormonal changes in the brain. Sex differences in schizophrenia have long been observed and established by epidemiological studies.[65,66] The average age at onset for women (25 years) is significantly later than in men (21 years), and there is a smaller peak of late first onset seen only in peri- and postmenopausal women after age 44. Male patients generally have more negative symptoms, a history of more maternal obstetric complications and poorer premorbid adjustment.[67] Even differences in anatomical brain abnormalities have been detected between male and female patients.[68] The greater severity of symptoms in males suggests that estrogen exerts a protective effect against schizophrenia.[66]

A number of susceptibility genes have been proposed to contribute to the etiology of schizophrenia, which is thought to have a strong heritable component. These genes include the serotonin transporter, the norepinephrine transporter, and the dopamine D2 receptor (**Table 2**). However, none of these genes has been unequivocally linked to or enables accurate prediction of disease. Lately, several strong candidate genes have emerged, including *COMT*,[49,51] *neuregulin*,[52] and *DISC1*[39] (see the section titled "Sex differences in mental disorders: depression and schizophrenia"). Studies directed specifically toward female patients have revealed additional susceptibility genes. Significant differences in the occurrence of polymorphisms in 2 genes, the dopamine D2 receptor and neurotrophin NT-3, have recently been observed between schizophrenic women and matched female controls.[45] Yet, no single gene has emerged as an overriding factor in schizophrenia; moreover, the physiological mechanisms underlying sex differences remain to be resolved. On the one hand, the disease is likely a canopy of different abnormalities with distinct phenotypes, and on the other, there appear to be multiple genes involved, each with low penetrance. Sex hormones modulate neurotransmitter signaling, including dopaminergic and serotonergic systems,[69-71] which could result in different susceptibility genes among male and female patients. This makes it difficult to resolve the genetic components of schizophrenia in male and female patients.

Atypical antipsychotics such as clozapine have become the treatment of choice for patients suffering from psychosis.[72,73] These medications are approved for acute and chronic management of patients with schizophrenia and are also widely used for other mental disorders with symptoms of psychosis, such a schizoaffective, bipolar, and depressive disorder.[74] While atypical antipsychotics interact with multiple receptor types, the primary mechanism of action is thought to involve serotonin 5-HT-2 and dopamine D2 receptors.[72] Each atypical antipsychotic has a distinct repertoire of target receptors. Multiple genes may play a role in disease susceptibility and drug response, but the contributions of specific genetic variations to disease remain unclear. The spectrum of genetic variants found in individuals may combine to allow for optimal selection of the antipsychotic drug. Furthermore, males and females may have a different spectrum of genetic variants underlying disease in the same ethnic population, which also could affect treatment outcome.

To address these issues, Arranz and colleagues[75] have measured a number of polymorphisms in multiple genes encoding neurotransmitter receptors and transporters in psychotic patients receiving clozapine, an effective atypical antipsychotic. A combination of 5 polymorphisms, in the serotonin receptors 5HT-2A and -2C, the histamine receptor H2, and the serotonin transporter SERT, improved the prediction of a positive response to clozapine to 77% accuracy. In principle, this type of analysis holds promise for predictive genotyping in antipsychotic therapy; however, predictability did not improve sufficiently to have direct impact on therapy, and moreover, the results may not ap

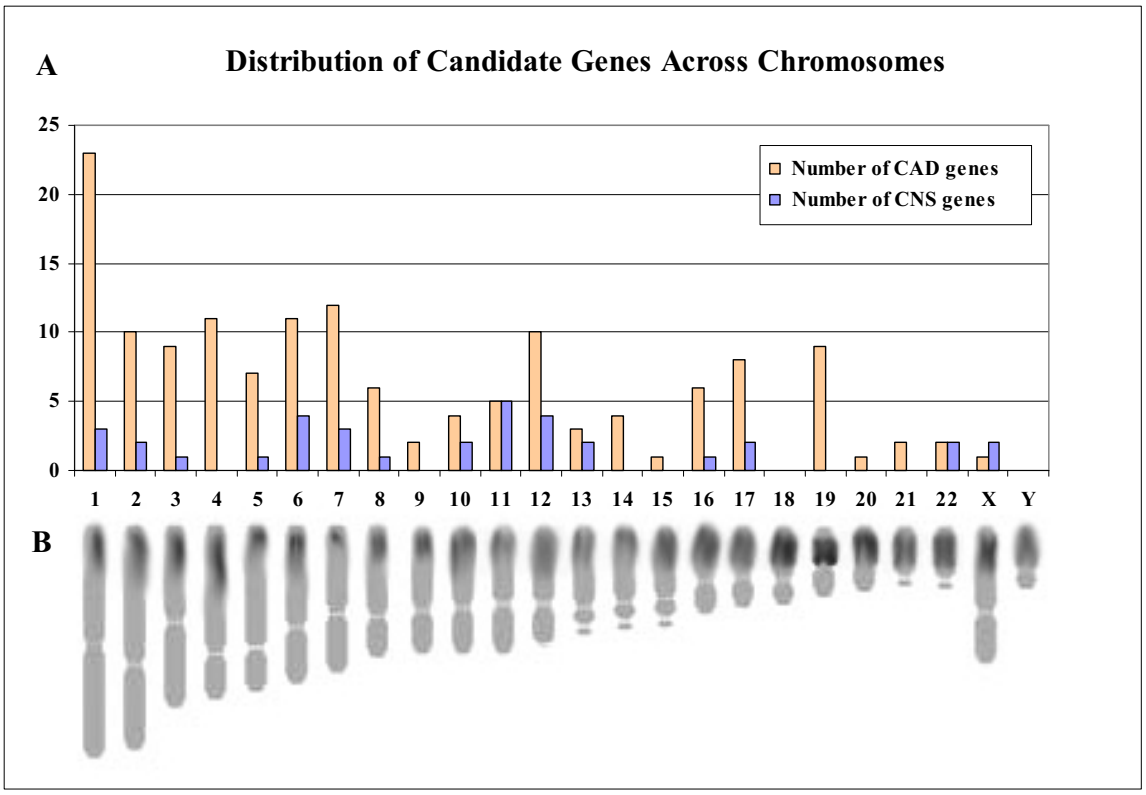

Figure 1. Distribution of candidate genes by chromosome (panel A) compared with the relative size of each chromosome (panel B).

ply to other patient populations. This lack of transferability is because there are substantial differences among ethnic populations in the prevalence of individual polymorphisms (SNPs) and varying linkage to functional genetic polymorphisms (population admixture). Several SNPs often are linked on the same chromosome and interact functionally. Phased SNPs are called haplotypes, which begin to supersede the use of single indicator SNPs in genetic association studies.[76,77] The inclusion of haplotype information holds promise for vastly improved power of such clinical studies and leads to the expectation that genetic risk factors will be unraveled in the future.

GENOMIC LOCALIZATION OF CANDIDATE GENES

To ascertain the genomic organization of candidate genes listed in **Tables 1 and 2**, we sorted them by their chromosomal position. **Figure 1** illustrates how candidate genes are distributed across the genome. Panel A is a graph of the number of genes implicated in each disease found on each chromosome. The schizophrenia and depression genes are listed together as cental nervous system (CNS). Panel B shows the size of each chromosome relative to the others to illustrate the scale of chromosomal size. It is striking that out of 144 CAD candidate genes, only a single gene *AGTR2*, the angiotensin II receptor AT2, is located on the X chromosome. Two CNS candidate genes out of 35 are also X linked: monoamine oxidase A (*MAOA*) and the serotonin 2C receptor (*HTR2C*). This scarcity of X-linked genes within the ranks of the candidate genes confirms that most sex differences in CAD, schizophrenia, and depression are not due to simple differences between the X and Y chromosomes, but most likely to secondary hormonal effects or other effects exerted by X and Y chromosomes. Many of the CAD genes are located in clusters on different chromosomes (**Tables 1 and 2**). This could reflect bias in the way these genes were identified as candidates (eg, if a region of a chromosome containing multiple genes was identified in an association study). One needs to consider that many of these candidate genes still await strong experimental confirmation. On the other hand, genes involved in similar pathways and processes often cluster together on a chromosome where they may share gene regulation and transcription factors. It is possible that multi-

ple members of a biological pathway are all candidates. Since hormones broadly regulate transcription, sex differences could be mainly due to global gene regulation by estrogens and other sex hormones.

SUMMARY

In the diverse disorders discussed here—CAD, schizophrenia, and depression—estrogen appears to play a prominent role, primarily as a protective agent. Even in depression, where women are more strongly affected than men, this may be due to a precipitous drop in estrogen levels, which happens, for example, after delivering a baby or during a menstrual cycle. Yet, multiple hormones are likely to act concomitantly, and hormonal levels are but one of the multiple changes induced by genomic differences between males and females. Our review points out the overriding need to consider sex in understanding disease and optimizing therapy, but it also highlights the complexity of multigenic disease and therapy. For CAD, we have evidence of genetic variants of some genes that contribute to disease progression and drug response in male patients but not in female patients, while other genetic variants are more important in women. Yet, these findings can only be the beginning of a systematic analysis of genetic factors and sex in complex disease. We have yet to define the most promising approaches capable of resolving these complexities such that our knowledge can lead to significantly improved drug therapy. With novel genomic technologies emerging at a frantic pace, we must now clearly define the problem in search of the best approach to understanding the genetics of disease and therapy.

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